PAPER ELECTROPHORESIS OF SERUM PROTEINS IN CHILDREN

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Since the description of paper electrophoresis in 1950 by Cremer and Tiselius and by Durrum among others, considerable interest has been shown in this cheap and relatively quick method of separating the various protein fractions. The serum proteins move through the fine cellulose mesh of the paper under the influence of an electric current, the separation of the various fractions being brought about by differences in their electrophoretic mobility at a selected pH. The bands are then shown up by staining with a protein dye. Usually five separate fractions are seen: albumin and four globulin fractions, designated α_1 , α_2 , β and γ (Fig. 1). The

FIG. 1.—Paper electrophoresis slip showing the five serum protein fractions. Protein molecules are negatively charged at pH 8.6 and move towards the anode.

albumin fraction is more or less homogeneous, but the various globulin fractions are heterogeneous and can be further subdivided by the use of other physical methods or biological techniques. The protein fractions obtained by paper electrophoresis approximate closely to those obtained by the original Tiselius method of electrophoresis in free solution. It is technically difficult, however, to estimate the various proteins quantitatively by the paper method (Wolstenholme and Millar, 1956).

As we wished to study protein changes in large numbers of children we decided not to attempt elution or scanning of the strips and to accept a visual comparison with normal controls as adequate for our purpose. The technique described by Flynn and de Mayo (1951) was used with a few minor

modifications. A slightly larger quantity of serum—namely, 0.02 ml.—than those authors used was applied to the paper from an accurately calibrated siliconed pipette, and we chose azocarmine B as the protein dye. When collecting blood samples by finger-prick care was taken to avoid haemolysing the specimen, because if free haemoglobin was present it appeared as a dense band in the $\alpha_2\beta$ region.

The aim of the investigation was to study the value of the method in paediatric hospital practice. Several reports on electrophoretic pattern changes in adults have been published, including those of Flynn (1954) and Penman (1956), but only one dealing specifically with sick children has come to our notice, that of Corbeel (1954), and his results were not very detailed.

Normal Patterns

To establish the normal pattern from birth to 12 years of age 130 paper strips were prepared from children without physical disease who attended the out-patient department. Average strips and those representing the extreme limits of normal for each year of age were mounted on cards and used as controls in the study of diseases. Bands which were outside the range of normal for the relevant age were interpreted as showing an increased or decreased intensity. The variations with age proved to be more or less identical with those described by Knapp and Routh (1949) using the classical Tiselius method. The most striking change is in the γ globulin, which at birth is at the adult level, but falls rapidly in the next three or four months until it almost disappears, only a faint stain being visible on the strip. Thereafter it gradually increases, but is still low at 1 year. By 2 years it has increased considerably, and between 6 and 10 years the adult level is again reached. The α_2 globulin band is well marked in the infant and gradually decreases to the adult level by about 12 years of age.

The albumin and α_1 and β globulin bands do not alter significantly with age (Fig. 2).

FIG. 2.—Normal serum protein electrophoretic patterns (Nos. 1-3) and those of various diseases (Nos. 4-20). Details are given in the table below.

abnormal. Early in the course of the disease the α_1 and α_2 globulin bands were increased, and later on the γ globulin became increased unless the infection had been very rapidly overcome. Out of 23 examples of acute infection, such as streptococcal tonsillitis, lobar pneumonia or bronchopneumonia, gastroenteritis and staphylococcal osteomyelitis, there were only two normal strips; 11 showed α_1 and α_2 globulin increases, and 10 a γ globulin increase as well. This type of response was seen also in eight out of nine infants under 1 year with acute infections. The γ globulin, normally low in this period, was capable of increasing in response to infection. Once the infection was overcome the protein patterns reverted to normal.

In subacute infections it was usual to find an increase in α_2 and γ globulins—for example, in active bronchiectasis or in fibrocystic disease of the pancreas or ulcerative colitis. Of 22 cases of fibrocystic disease, nine showed α_2 and γ globulin increases and seven showed γ globulin increases only; five were normal and one had a low γ globulin. Of eight cases of ulcerative colitis, five showed α_2 and γ globulin increases and three showed γ globulin increases only. Two early cases showed α_1 and α_2 globulin increases only. In three cases of Caffey's disease (infantile cortical hyperostosis) α_2 and γ globulin increases occurred which after several months returned to normal. This suggests that there

KEY TO SERUM PAPER ELECTROPHORETIC PATTERNS IN FIG. 2

Number	Sex	Age	Disease	Pattern Changes				
				A	α_1	α_2	β	γ
1	M	1 day	Normal					
2	M	4 mos.	Normal					
3	F	20 mos.	Normal					
4	M	7 yrs.	Acute tonsillitis		+	+		+
5	F	5 yrs.	Fibrocystic disease		+	+		+
6	F	3 yrs.	Chronic pulmonary tuberculosis					+
7	M	2 yrs.	Agammaglobulinaemia					+
8	F	10 yrs.	Acute rheumatic fever		+	+		+
9	M	2 yrs.	Still's disease		+	+		+
10	M	12 yrs.	Rheumatoid arthritis			+		+
11	M	4 yrs.	Acute nephritis			+		+
12	M	4 yrs.	Nephrotic syndrome			+	+	+
13	M	4 yrs.	Nephrotic syndrome during treatment			+	+	+
14	M	7 yrs.	Infective hepatitis			+		+
15	M	2 mos.	Neonatal hepatitis			+		+
16	F	10 mos.	Congenital obliteration of bile ducts		+			+
17	M	10 yrs.	Cirrhosis					+
18	F	8 yrs.	Haemosiderosis					$\gamma_1 + \gamma_2 +$
19	F	16 mos.	Liver disease with hypercholesterolaemia					$\gamma_1 + \gamma_2 +$
20	F	8 mos.	Idiopathic hypercalcaemia of infants					$\gamma_1 + \gamma_2 +$

Abnormal Patterns

Infections. No change in electrophoretic pattern was detected in mild infections such as colds, bronchitis or low-grade urinary infections. However, where fever, leucocytosis and constitutional disturbance were present the proteins were usually

is an inflammatory basis to Caffey's disease.

In chronic infections the γ globulin only was raised, for example, in six cases of chronic tuberculosis.

The serum of seven out of 14 children with a history of constantly recurring infections showed

TABLE 1

COMPARISON OF SERUM PROTEIN ELECTROPHORESIS FINDINGS IN RHEUMATIC FEVER, STILL'S DISEASE, RHEUMATOID ARTHRITIS AND ACUTE NEPHRITIS

Condition	Total Number of Cases	α_1 , α_2 and γ Globulin Raised	α_2 and γ Globulin Raised	γ Globulin Raised	Normal Pattern
Rheumatic fever	20	5	4	7	4
Still's disease	8	6	1	1	0
Rheumatoid arthritis	7	0	2	2	2
Average E.S.R. (mm./1 hr.)	—	51	44	35	13
Range of E.S.R. (mm./1 hr.)	—	11-80	12-112	6-95	6-24
Acute nephritis*	32	0	12	3	12

* In three cases γ globulin was low; in two α_2 globulin only was raised.

normal protein patterns, while in three cases there were α_2 and γ globulin increases of the order already noted in acute and chronic infections. Two showed a low γ globulin, a condition one might term 'hypogammaglobulinaemia'; in the remaining two no γ globulin at all was visible on the strip. In these cases the diagnosis of 'agammaglobulinaemia' was confirmed by the classical Tiselius technique, as the γ globulin concentration was less than 0.1 g. per 100 ml. It is of considerable interest that in a third case of agammaglobulinaemia there was no history of recurrent infections and the child was examined by us because he had rheumatoid arthritis (a condition which we were studying at the time) with typical bilateral involvement of knees and ankles. The association of rheumatoid arthritis with agammaglobulinaemia is unexpected in view of the hypogenesis of reticulo-endothelial tissue associated with the latter condition.

Very high γ globulin levels were found in three children with severe chronic staphylococcal infections, two with empyema and one with enteritis. Janeway, Craig, Davidson, Downey, Gitlin and Sullivan (1954) described this condition in a group of infants, calling it 'hypergammaglobulinaemia' as the γ globulin level was higher than that seen in the usual chronic infection. The titre of antibodies was high in their cases, so that the failure to overcome the infection speedily lay elsewhere than in the immunity mechanism.

Rheumatic Fever, Still's Disease and Rheumatoid Arthritis. The rheumatic diseases were selected for study because a rise in total globulin commonly occurs in these conditions, but it soon became apparent that the globulin patterns were in no sense diagnostic and resembled closely those seen in subacute and chronic infections. When tests were made within a day or two of the onset of rheumatic symptoms the γ globulin level was always raised, a finding which was helpful on one occasion in differentiating acute osteomyelitis from rheumatic

fever, as the γ globulin level is not raised in the early stages of acute osteomyelitis. Twenty children with rheumatic fever were studied, eight with Still's disease (joint swellings, adenopathy and splenomegaly, with or without rash or pericarditis) and seven with rheumatoid arthritis (arthritis being the only clinical manifestation of disease). The results obtained when the children were first seen are shown in Table 1. A rise of α_1 , α_2 and γ globulins occurred in the most acute cases of rheumatic fever, three of which had pericarditis, and in children with the widespread tissue involvement of Still's disease. Changes in α_2 and γ globulins, or γ globulin increases alone, were found in the less acute cases of rheumatoid arthritis and in the later stages of rheumatic fever and Still's disease. With clinical improvement the α_2 globulin fell to normal before the γ globulin. Children showing normal patterns were, without exception, very mildly affected or had passed the acute phase of illness before coming to hospital.

As with simple infections, the more acute cases tended to have higher erythrocyte sedimentation rates (E.S.R.). Great variation, however, occurred and, although the average E.S.R., as seen in the table, worked out in descending order as the cases decreased in acuteness, the range of E.S.R. was almost the same in each group with abnormal globulins. In assessing the stage and severity of rheumatic fever, Still's disease and rheumatoid arthritis, therefore, it may be an advantage to take the electrophoretic findings as well as the E.S.R. into consideration. This may be particularly useful in cases with cardiac failure.

When followed throughout the course of these disorders the E.S.R. and electrophoretic pattern did not run parallel (Table 2). The tests were performed weekly on the same blood sample. Out of nine cases of rheumatic fever thus followed, four showed abnormal electrophoretic patterns for weeks or even months after the return of the E.S.R. to 10 mm. in one hour or less. In two cases, on the other hand,

TABLE 2

RELATION BETWEEN RETURN TO NORMAL OF SERUM PROTEIN ELECTROPHORESIS PATTERN (S.P.E.) AND E.S.R.

Conditions	Total Number of Cases	S.P.E. and E.S.R. Normal Together	S.P.E. Normal before E.S.R.	E.S.R. Normal before S.P.E.
Rheumatic fever ..	9	3	2	4
Still's disease and rheumatoid arthritis ..	6	5	1	0
Acute nephritis ..	10	4	6	0

the electrophoretic pattern became normal before the E.S.R., and in three the tests corresponded exactly. So few cases of rheumatoid arthritis had returned to normal at the time of assessment that the numbers are too small to be of much value.

To summarize, the changes in serum protein electrophoretic pattern which may be found in rheumatic fever, Still's disease and rheumatoid arthritis are of no help diagnostically and closely resemble those seen in simple infections. Electrophoresis may, however, be a useful adjunct to determination of the E.S.R. in assessing the stage and severity of the disorder and in deciding how long to continue treatment, whether by cortisone, salicylates or bed rest.

Acute Nephritis. In view of the aetiological similarity between acute nephritis and the rheumatic conditions, it was of interest to find how closely the patterns resembled each other in the two groups. The study covered 32 children with acute nephritis (see Table 1). None showed the α_1 , α_2 and γ globulin increases seen in the most acute rheumatic conditions; 12 showed an α_2 and γ globulin rise, two an α_2 globulin rise only, while in three there was a slight increase and in three a slight decrease in γ globulin. Twelve showed a normal pattern, but nine of these children had passed the most acute phase when first seen. Consecutive studies (Table 2) showed that in four out of 10 cases the E.S.R. and serum protein electrophoretic pattern returned to normal together, while in six cases the electrophoretic pattern returned to normal first. Usually, but not invariably, the albumin disappeared from the urine before the results of blood tests became normal.

Of five cases of chronic nephritis of long standing with uraemia, four showed a slight reduction of γ globulin.

Nephrotic Syndrome. Changes in serum proteins in the nephrotic syndrome are dramatic and well recognized, the fall in γ globulin being in contrast to the rise seen in the acute stage of nephritis. The

electrophoretic pattern is diagnostic with a low albumin, an $\alpha_2\beta$ complex, the two bands having been replaced by one broad deeply staining band, and a low γ globulin; the latter disappears in the most severe cases to give an acquired agammaglobulinaemia. In milder cases a pattern which might be termed 'partially nephrotic' occurs: the albumin is slightly reduced, the α_2 band is broader and more densely stained than normal, and the γ globulin is reduced. This partial pattern, which we first recognized in nephrotic patients improving under hormone therapy, was of value in assessing the cause of symptomless albuminuria in three cases. These children gave no history of oedema and had no urinary infection, their albuminuria being discovered on routine testing. In two the serum cholesterol was raised. None showed a reversal of albumin:globulin ratio, as the rise in α_2 globulin was offset by the fall in γ globulin. In each case the blood urea and an intravenous pyelogram were normal. One progressed to classical nephrosis, but in the other two the electrophoretic patterns returned to normal with disappearance of the albuminuria without treatment, and these children might be considered to have suffered *formes frustes* of the nephrotic syndrome. When stains for lipid or cholesterol are used, increased amounts of both fat and cholesterol are seen in the $\alpha_2\beta$ region.

In 25 cases of nephrosis a serial study of the pattern changes during hormone therapy was made. At the time of the disappearance of albuminuria the pattern began to alter. The $\alpha_2\beta$ complex separated into α_2 and β bands; later on the albumin re-formed, and finally after several weeks the γ globulin returned to normal. The serum cholesterol level fell to normal at about the same time as the γ globulin regenerated. The final return of the γ globulin to normal was used in determining the duration of full hormone therapy; the periods of treatment required to achieve it varied from case to case and did not always correspond with the apparent initial severity. With other forms of treatment, such as with resins, urea or mercurial diuretics, resulting in loss of oedema, but not to disappearance of albuminuria, no improvement in the nephrotic pattern was seen. When relapse occurred the first abnormality noted was the reappearance of albuminuria, and only after a week or so did the serum protein picture alter. This is consistent with present concepts of serum protein synthesis and storage.

Liver Disease. Alteration in serum proteins is the basis of the turbidity tests—for example, the thymol turbidity, Takata-Ara and Kunkel tests, the last being largely dependent on an increase in

TABLE 3
SERUM PROTEIN ELECTROPHORESIS CHANGES IN CERTAIN LIVER DISEASES

Disease	Total Number of Cases	α_1 Globulin		α_2 Globulin		β Globulin		γ Globulin		Normal Pattern
		—	+	—	+	—	+	—	+	
Infective hepatitis	21	0	0	2	3	1	1	0	3	14
Neonatal hepatitis	7	0	0	2	0	2	0	0	0	4
Congenital obliteration of bile ducts	8	0	4	0	0	0	0	0	3	3
Cirrhosis	15	0	0	5	0	0	0	0	11	2

γ globulin. In infancy these tests are notoriously unreliable, and this may be because of the difference in globulin pattern normally seen at this age, particularly the low γ globulin. By comparing the electrophoretic patterns of jaundiced babies with those of normal infants it was hoped that more valuable information might be obtained than from the turbidity tests alone. However, the results, although useful in some cases, were by no means uniform.

Of 21 cases of infective hepatitis in childhood (Table 3), 14 showed normal strips, three a rise in α_2 and β globulins, two a reduction in α_2 globulin, one a raised β globulin and one a lowered β globulin. From seven cases of neonatal hepatitis, four strips were normal, one showed a low $\alpha_2\beta$, one a low α_2 and one a low β globulin. Of eight cases of congenital obliteration of the bile ducts, proved at operation or necropsy, three showed normal strips, three an increase in α_1 and γ globulins, and one an increase in α_1 globulin only; the other showed variable changes in α_2 and β globulins. In 15 cases of cirrhosis only two strips were normal, and 11 showed a rise in γ globulin. Of these, two cases of haemosiderosis showed a dense band filling in the space between the β and γ globulins, an increase in the so-called γ_1 globulin. In four cases the α_2 globulin was low in association with a raised γ globulin, and in one the α_2 globulin was low with a normal γ globulin. Of 16 cases diagnosed incompletely as 'hepatosplenomegaly', 12 were normal. In three the α_2 globulin was low and the γ globulin raised, a pattern seen in cirrhosis of the liver. In one interesting case of galactosaemia in an infant the depression of α_2 and β globulins seen on the strip reverted to normal a week after the introduction of a lactose-free diet. In two cases of hepatic coma the α_2 and β globulins were low and the γ globulin high. Two rather unusual cases with jaundice and hypercholesterolaemia in which no liver biopsy was performed showed marked α_2 and β globulin increases.

In reviewing the changes in liver disease certain general patterns were apparent. The α_1 globulin was increased only in congenital obliteration of the

bile ducts, and the α_2 globulin only in infective hepatitis. A decrease in α_2 globulin was a fairly common finding and was seen in other cases of infective hepatitis, in neonatal hepatitis and in cirrhosis. Changes in β globulin were rare and variable. A rise in γ globulin was almost the rule in cirrhosis, and occurred occasionally in infective hepatitis and in congenital obliteration of the bile ducts.

Idiopathic Hypercalcaemia of Infants. A study was made of 18 infants with idiopathic hypercalcaemia, but 10 of them had already passed the acute phase. In eight the serum calcium level was still raised, and in four of these there was a very definite increase in α_2 and β globulins, similar to the pattern seen in the two cases of liver disease with hypercholesterolaemia mentioned above. Two strips were also stained for fat and showed a rise in β lipoprotein concentration. One infant showed a rise in α_2 and γ globulins, and one a rise of γ globulin only. The other two infants with an increased blood calcium had a normal pattern. Two of the 10 infants with a normal serum calcium level by the time of examination showed abnormalities in the α_2 and β band persisting beyond the hypercalcaemic phase—a finding of some interest.

Miscellaneous Conditions. Strips were prepared from nearly 200 children in hospital, but no changes of diagnostic value were found. Conditions investigated included, among many others, the following: malignant tumours, leukaemia, Hodgkin's disease, haemolytic anaemia, histiocytosis, Gaucher's disease, thyrotoxicosis, cretinism, coeliac disease and pink disease.

Discussion

Five serum protein bands have been studied by paper electrophoresis. The albumin band is homogeneous or nearly so, but the other bands are mixtures of several proteins (Table 4). It will be seen that many specific proteins have been isolated from these fractions, although the α_2 globulin band is still largely unidentified. Some proteins are

TABLE 4
PROTEIN COMPONENTS OF PLASMA (ADAPTED FROM
HUGHES, 1954)

Electrophoretic Band	Known Components	Concentration in Plasma (g./100 ml.)
Albumin	Crystalline albumins	4.30
α_1 Globulin	α_1 Mucoprotein	0.30
	α_1 Lipoprotein	0.05
α_2 Globulin	Ceruloplasmin	0.20
	α_2 Lipoprotein	0.70
	Complement	0.03
β Globulin	Transferrin	0.02
	β Lipoprotein	—
γ_1 Globulin	Fibrinogen	1.10
	Antihæmophilic globulin	0.25
	Isohaemagglutinins	0.40
γ Globulin or γ_2 Globulin		0.50
		0.30
		—
		—
	Diphtheria antitoxin	0.90
		0.001
Total plasma protein		7.80

present in such low concentration that they are not likely to contribute much to the fairly gross quantitative levels required for visual appreciation; for example, it would appear that the increase in γ globulin found in chronic infections is greater than could be accounted for by a rise in specific antibodies, as they form such an infinitesimal part of the γ globulin total. The function of the apparently inert proteins in the various fractions is unknown, and their presence may be masking changes of specific active proteins occurring in disease. With the further identification of serum proteins diagnostic changes may be discovered which are not revealed by the present rather crude methods. To date only nephrosis, agammaglobulinaemia and multiple myeloma have pathognomonic patterns. The changes in infections, rheumatic conditions and liver disease may occasionally be of help in diagnosis, but, on the whole, their chief value lies in following the return to normal during the course of treatment.

The disorders which may be associated with an increase or decrease in the various globulin bands are shown in Table 5. Consideration of these may help to clarify the interpretation of the protein changes found in disease in childhood.

Summary

The normal serum protein patterns obtained by paper electrophoresis in childhood have been described. Assessment of abnormal paper strips by visual comparison with the range of normal for the age was considered adequate for clinical purposes.

Changes occurring in a variety of conditions,

TABLE 5
DISEASES WHICH MAY BE ASSOCIATED WITH GLOBULIN
CHANGES

Globulin	Decreased	Increased
α_1	None	Acute infections Rheumatic fever Still's disease Congenital obliteration of bile ducts
α_2	Infective hepatitis Neonatal hepatitis Cirrhosis Galactosaemia	Acute infections Subacute infections Rheumatic fever Still's disease Rheumatoid arthritis Acute nephritis Nephrotic syndrome Infective hepatitis Liver disease with hypercholesterolaemia Idiopathic hypercalcaemia of infants
β	Infective hepatitis Neonatal hepatitis Galactosaemia Hepatic coma	Nephrotic syndrome Infective hepatitis Liver disease with hypercholesterolaemia Idiopathic hypercalcaemia of infants
γ	Hypogammaglobulinaemia Agammaglobulinaemia Chronic nephritis with uraemia Nephrotic syndrome	Subacute infections Chronic infections Rheumatic fever Still's disease Rheumatoid arthritis Acute nephritis Infective hepatitis Congenital obliteration of bile ducts Cirrhosis Hepatic coma

including simple infections, rheumatic affections, acute nephritis, nephrosis, liver disease, and idiopathic hypercalcaemia, have been discussed.

The method of paper electrophoresis has only limited diagnostic value, but it may provide a useful additional guide to progress in the rheumatic conditions, nephrosis and diseases of the liver in particular.

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